From: Lynne Harris
Sent: Monday, August 12, 2002 4:39 PM
To: 'scott masten'
Subject: Comments on Federal Register Notice No. 113, ISSN 1521-9402, June 12, 2002
Importance: High

August 12, 2002

Mr. Scott Masten
NIEHS/NTP
P. O. Box 12233
MD-A3-07
Research Triangle Park, NC 27708

re - Federal Register Notice No. 113, ISSN 1521-9402, June 12, 2002, Chemical Testing Toxicology Program Seeks Comments on Whether It Should Test 19 Substances

Dear Mr. Masten:

In response to the referenced Federal Register Notice, the Organic Peroxide Producers Safety Division (OPPSD) of the Society of the Plastics Industry, Inc (SPI) is providing its recommendation on testing Tert-Butyl Hydroperoxide, CAS No. 75-91-2 as well as the information requested on production levels, human exposure, environmental occurrence and public health. The OPPSD recommends against testing the Tert-Butyl Hydroperoxide, CAS No. 75-91-2 because of the reasons stated below.

Rationale Against Testing TBHP

Tert-butyl hydroperoxide (TBHP) is a high production volume chemical that has been selected for Risk Assessment by the European Union under the auspices of the Existing Substances Regulation (793/93). This activity is being managed by the Netherlands Competent Authority, which has responsibility for preparing the first draft of the Risk Assessment report prior to its review, modification and endorsement by the other EU Member States. The Netherlands has some familiarity with this substance, having sponsored its inclusion in the OECD-SIDS program in the 1990's.

During the SIDS review a combined oral repeat dose and reproductive/developmental toxicity screening study (OECD 422 guideline) and an *in vivo* mouse micronucleus test (OECD 474 guideline) were conducted. In the OECD 422 investigation, there were no obvious treatment-related adverse effects on reproductive or developmental parameters in rats given 3, 10 or 30 mg/kg body weight/day for up to 45 days, while toxicological sequelae were limited to increased accumulation of tubular proteinaceous material and tubular nephrosis in kidneys from mid- and high dose male (but not female) Wistar rats. The laboratory conducting the study concluded that the histopathological characteristics of these changes were consistent with a2m-globulin nephropathy (Jonker et al 1993). Results from the micronucleus test were negative after intravenous injection of the maximum tolerated dose of 100 mg/kg body weight (Delft van et al 1995).

The EU Risk Assessment program for TBHP is currently on-going and additional studies have been initiated.

In a meeting held January 2001, the Dutch Authorities noted that the *in vitro* genotoxicity profile of TBHP, coupled with 'positive' results from a mouse skin painting study (Hoshino et al 1970) raised concerns over the carcinogenic potential of TBHP. Industry expressed doubts over the reliability of the results from skin painting, which is both non-guideline and non-GLP compliant. Also, industry noted that the reported increase in malignant and benign skin tumors was only seen when TBHP in benzene was applied after dermal initiation with 4-nitroquinoline-N-oxide, and that there was no equivalent control group to establish the effects of 4NQO and benzene. This is considered to be an important omission, given the possibility that repeated inflammation/irritation may have triggered tumor formation through an epigenetic mechanism. It was also suggested that the lack of effect seen in the micronucleus study indicated that *in vivo* detoxication processes effectively limited the genotoxic potential of TBHP.

To help put these findings in perspective, the Dutch authorities and industry agreed to study the fate of TBHP in the body. Results from the preliminary GLP-complaint, method development study are complete and show:Good absorption and recovery of radiolabel in the rat after oral (gavage) administration or sub-cutaneous injection (10 or 100 mg/kg bwt), with highly comparable blood kinetics, tissue retention and urinary metabolite profiles for both routes;

- Very poor mass balance recovery of radiolabel following dermal application of aqueous TBHP to clipped rat skin. The laboratory concluded that significant (perhaps 90%) evaporative loss had occurred during the short time it took to apply the test substance to the skin and 'close' the dermal exposure chamber;

Based on these findings, the design of the main study is under discussion with the Dutch Authorities and the EU Member States. It is probable that this work will commence in the fourth quarter of 2002 and take around 6-8 months to complete.

In addition mechanistic work to understand the basis of the renal lesions noted in the OECD 422 study is also being conducted. This includes immunostaining with antibody specific for a2m-globulin, and possibly other techniques to quantify the amount of a2m-globulin present in male rat kidney after exposure to TBHP. This program is still being developed with the laboratory retained to do the work, and results are expected in 2003.

The above described metabolism and mechanistic studies will improve the scientific knowledge on the carcinogenic potential of TBHP. The study results may help to address the uncertainties raised regarding TBHP carcinogenicity in the EU Risk Assessment or suggest the need for further study and testing.

References:

Delft van JHM, Vogel de N (1995). Micronucleus test with tertiary butyl hydroperoxide-70 in mice. TNO Project No 450042-004. Report No V95.574, October 1995 (unpublished).

Hoshino H, Chihara G, Fukuoka F (1970). Detection of potential weak carcinogens and procarcinogens II. Carcinogenicity of tertiary butyl hydroperoxide. Gann 61 121-124.

Jonker D, Waalkens-Berendsen DH, Wijnands MVW (1993). Range-finding studies and combined repeat dose oral and reproductive/developmental toxicity screening test with an aqueous solution of hydroperoxide, 1,1-dimethyl (Aq TBHP-70) in rats. TNO Report No V92.494, January 1993 (unpublished).

In the 1997, the OPPSD pursued a voluntary testing program to learn if a screening method could be developed that would determine the potential of newly synthesized organic peroxides to produce carcinogenic or tumor inititiating activity instead of the conventional two-year bioassay test. A study was contracted to Dr. Thomas Slaga, then with the M.D. Anderson Cancer Center and currently Director of the AMC Cancer Research Center in Denver, Colorado. The study evaluated the potential of nine organic peroxides as well as hydrogen peroxide to produce DNA damage and sustained epidermal hyperplasia in Sencar mice. A copy of this study was recently sent to NTP by the EPA Interagency Testing Committee. The study results indicated that organic peroxides are not tumor initiators or complete carcinogens. The U.S. EPA has previously reviewed the test data (Risk Assessment Division, OPPT) and concluded that this study as well as available information "does not support the continued identification of peroxides as a new chemical category presenting concerns for possible carcinogenicity." For your information a summary that OPPSD provided to the US EPA on the screening method is attached to these comments.

Additionally, the Consumer Healthcare Products Association recently conducted dermal oncogenicity studies on rats and mice with benzoyl peroxide for the FDA and found that benzoyl peroxide lacks carcinogencic potential. Abstracts of the studies were presented at the March 2000 Society of Toxicology Meeting (see attached abstracts).

TBHP-PRODUCTION AND EXPOSURE

The production level for TBHP on a neat basis in 1998 for the OPPSD members worldwide was approximately 19 million kg.

TBHP can be manufactured by several different routes. The route of greatest commercial significance is by direct reaction of oxygen with isobutane (2-methylpropane). Purification is accomplished by azeotropic distillation to yield a product nominally of 70% concentration in water. This manufacturing process is done in closed systems, and release of TBHP is only via fugitive emissions.

Alternatively, TBHP may be produced by reaction of t-butyl alcohol (2-methylpropan-2-ol) with hydrogen peroxide.

To the best of OPPSD member company knowledge, TBHP is used only as a chemical intermediate. The largest volume use is the oxidation of propylene (propene) to propylene oxide. Other uses are as a raw material for the production of other organic peroxides and as an initiator for polymerization reactions. In all cases the TBHP is reacted to extinction.

OPPSD is not aware of any use of TBHP as an unreacted ingredient in consumer products.

Exposure of the general population to TBHP is thus expected to be essentially zero. Exposures are generally limited to chemical plant workers only. TBHP is transported by bulk tank truck and in 55-gallon drums and some smaller containers. Thus, there is a small risk of exposure to transporters as well. One major TBHP manufacturer has done some limited task exposure monitoring of its plant workers. Measured task exposures for workers performing routine tasks ranged from 0.01-5 ppm. One exposure for a worker performing a non-routine task was measured at 16 ppm.

Thus, the US EPA's evaluation of the carcinogenic potential of TBHP and the minimal human exposure to this substance strongly suggest that TBHP is not carcinogenic and further carcinogencity testing is not warranted. Additionally, the ongoing work in the European Union to investigate the toxicokinetics and mechanisms of toxicity of TBHP should provide additional useful information. OPPSD believes that NTP should reconsider testing TBHP as current and EU data should suffice, or NTP should at least delay consideration of testing until after ongoing EU testing is complete.

If you have any questions or require additional information, please contact me by phone at 202-974-5217 or by e-mail at lharris@socplas.org.

Sincerely,

Lynne R. Harris

Executive Director, Organic Peroxide Producers Safety Division

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SUMMARY OF OPPSD RESEARCH PROGRAM

In order to address the EPA's carcinogenicity concern for organic peroxides, the Organic Peroxide Producers Safety Division (OPPSD) has recently completed a research program demonstrating organic peroxides are not complete carcinogens or tumor promoters. Based on these data, the EPA removed the cancer category of concern for organic peroxides in May 2001.

A screening assay to assess the carcinogenicity potential of organic peroxides was developed in the laboratory of Dr. Thomas Slaga, a well known carcinogenicity expert. Briefly, Sencar mice were dermally exposed for 4 weeks to 9 representative organic peroxides (including 2 hydroperoxides but not t-butyl hydroperoxide), as well as, positive and negative controls. Markers of tumor promotion, as well as, mutations in specific codons of the Hras oncogene, an oncogene associated with carcinogenicity, were evaluated. Mutations were not detected after organic peroxide exposure. Phase II was conducted to determine if the methods used were sensitive enough to detect mutations in the Hras oncogene after exposure to weak carcinogens. Phase II included the evaluation of the two organic peroxides with the strongest response as tumor promoters, t-butylperoxy benzoate (TBPB) and p-t-butyl isopropylbenzene hydroperoxide (TBIBHP), as well as an additional positive control, urethane – a weak carcinogen. Following treatment for 12 weeks, the positive controls exhibited the anticipated positive response. TBPB and TBIBHP did not induce an increase in mutations at codons 12, 13 or 61. The authors concluded that TBPB and TBIBHP do not possess tumor initiating activity and that the results from the positive controls indicate that the assay is sensitive enough to detect weak carcinogens.

EPA reviewed the results from both studies and concluded that the data "does not support continued identification of peroxides as a new chemical category presenting concerns for possible carcinogenicity". Given that a hydroperoxide (TBIBHP) was tested in this assay and EPA has dismissed its concerns about the potential carcinogenicity of peroxides as a class of chemicals, OPPSD would like to suggest that NTP consider removing TBHP from its list of chemicals to be tested.

INTRODUCTION

The Organic Peroxide Producers Safety Division (OPPSD) conducted a research program to develop a screening assay to be used to evaluate the carcinogenicity potential of organic peroxides. This assay was developed in the laboratories of Dr. Tom Slaga, a well-recognized expert in the field of carcinogenicity. All protocols were reviewed and approved by EPA prior to initiation of the studies.

Phase I included the evaluation of 10 organic peroxides in a battery of short term assays including mutation in the Hras oncogene. Phase II assessed the sensitivity of this

screening assay and confirmed the negative results of the organic peroxides by testing the 2 organic peroxides of highest concern to EPA.

PHASE 1 SUMMARY

The objective of Phase I was to develop a short term screening assay to evaluate the carcinogenic potential of organic peroxides. Nine organic peroxides representing several classes of organic peroxides (see below) hydrogen peroxide were evaluated in a battery of short-term assays including mutation in the Hras oncogene (codons 12, 13 and 61), dermal thickness, epidermal thickness, dermal inflammation, and oxidative DNA damage.

Benzoyl peroxide (BZP) 94-36-0
Di-t-butyl peroxide (DTPB) 110-05-4
t-butyl perbenzoate (TBPB) 614-45-9
p-t-butyl isopropylbenzene hydroperoxide (TBIBHP) 6285-32-1
cumene hydroperoxide (CHP) 80-15-9
dicetyl peroxydicarbonate (DPD) 263222-14-5
Hydrogen peroxide (H2O2) 7722-84-1
Methyl ethyl ketone peroxide (MEP) 1338-23-4
t-butyl O-(2-ethyl-hexylmonoperoxycarbonate) (TBEC) 3443-12-4

Controls
7,12-dimethylbenz(a)anthacene (DMBA) 57-97-6 – positive control
Acetone ACT 67-64-1 – vehicle control
12-o-tetradecanoyl phorbol-12 acetate (TPA) 15651-29-8 – tumor promoter control
Ethanol (ETOH) 64-17-5 – vehicle control
Dimethyl phthalate (DMPH) 131-11-3 – vehicle control

None of the peroxides induced mutations in the Hras oncogene although some produces increases in the other parameters. DMBA produced mutations in codon 61 of the Hras oncogene.

PHASE II SUMMARY

The objective of Phase II was to assess the sensitivity of the screening assay.
At EPA's suggestion, Phase II was initiated using the appropriate positive controls for the Hras mutations, as well as repeating the two organic peroxides of most concern to EPA (TBIBHP, TBPB).

The screening assay was designed to be very sensitive.

- The strain and species of animals used, the SENCAR mouse, has been shown to be very sensitive to skin cancer.
- In addition, the two organic peroxides [TBPB (t-butyl peroxybenzoate) and TBIBHP (p-t-butylisopropylbenzene hydroperoxide)] tested were those of

highest concern to EPA, particularly because they were positive for all endpoints with the exception of mutations to the Hras oncogene in the Phase I study.

- The highest dose group for TBPB was the highest dose that did not result in excessive skin irritation during a 2-week range finding study. The high dose group of TBIBHP had comparable irritation as the high dose TBPB group.

Based on EPA recommendations, Phase II of the OPPSD research program was designed to assess the sensitivity of the screening method using the following positive controls for mutations in the specific codons:
Urethane and dimethylbenz(a)anthracene for codon 61,
Benz(a)pyrene for codon 13
N-methyl-N-nitrosoguanidine for codon 12

The positive controls produced the expected results. During the recent meeting between OPPSD and EPA, EPA expressed concern regarding the weak response noted with urethane alone compared with urethane with TPA and questioned what results would be obtained if TPA was used as a promoter after organic peroxide administration. The OPPSD study included parameters to assess promoting activity including inflammation, dermal hyperplasia and epidermal hyperplasia. The high dose organic peroxide groups exhibited promoting activity (see attached graphs), to a similar extent as TPA. So while TPA was not used as a promoter with the organic peroxides, there was promotion in the high dose organic peroxide groups.

Urethane is considered a weak carcinogen. The available data indicate the dose of urethane used in the present study (670 nmol) does not produce tumors without the addition of TPA (IARC 1974). This dose of urethane did result in mutation in codon 61 after 12 weeks of exposure with and without TPA indicating the methodology used was capable of detecting weak carcinogens.

Similar results have been noted with DMBA. High doses are considered strong carcinogens, whereas, lower doses may act as an initiator. The high dose DMBA group exhibited mutations on codon 61 after 4, 8, and 12 weeks of exposure. The low dose of DMBA in this study does not produce tumors without the addition of TPA yet caused mutations in codon 61 after both 8 and 12 weeks of exposure.

The results described above indicate the methodology used is sensitive enough to detect weak carcinogens. The results also indicate organic peroxides are not cancer initiators or complete carcinogens.

ADDITIONAL SUPPORTING DATA

Genetic toxicity studies have been conducted on many organic peroxides (both published and non-published data). In some instances, positive genotoxic results have been obtained *in vitro*, particularly with the Ames bacterial mutagenicity assay. However, *in vivo* studies such as the micronucleus have been negative.

Several studies can be found in the literature that indicate some organic peroxides can act as tumor promoters. However, only a few studies have indicated organic peroxides may be carcinogens. These are older studies, often with serious experimental design flaws that make interpretation impossible. Most importantly, in the instances where the studies have been repeated, positive carcinogenic responses have NOT been duplicated.

In addition, benzoyl peroxide has recently been re-evaluated by IARC. This 1999 review included the study by Kurakawa. IARC has maintained benzoyl peroxide as a Group 3, not classifiable as to its carcinogenicity to humans. In Dr. Lai's review, he questioned IARC's 1987 classification because it did not include the Kurakawa study. The 1999 IARC review is complete and indicates that benzoyl peroxide is not an anticipated animal or human carcinogen.

The Consumer Healthcare Products Association (CHPA) has completed a 2-year skin carcinogenicity studies with benzoyl peroxide in mice and rats (see attached SOT abstracts). Similar to the OPPSD studies, skin irritation was used to determine the maximum tolerated dose. These new studies indicate dermal exposure to benzoyl peroxide does not produce skin tumors in rats or mice, even at concentrations high enough to produce extensive skin irritation.

CONCLUSIONS

The data from the research program conducted by OPPSD demonstrate organic peroxides are not complete carcinogens or cancer initiators. This conclusion is supported by the recent studies by CHPA and the recent review of benzoyl peroxide by IARC.

In addition, the results of the current study indicate the screening assay can detect mutations in codons 12, 13, and 61 of the Hras oncogene after 12 weeks of exposure. In addition, the assay is sensitive enough to detect weak carcinogens as noted by the responses in the low dose DMBA and urethane alone groups.

The available data were sufficient for EPA to remove the cancer category of concern for organic peroxides, as stated in their May, 2001 letter to OPPSD (see attached).

References:

SPI 1996. Determination of the Potential of Organic Peroxides to Induce Sustained Skin Hyperplasia and DNA damage. Report Spi/SPRD 96-1.

SPI. 2000. Determination of the Potential of Organic Peroxides to Induce Sustained Skin Hyperplasia and DNA Damage: Phase II Assessment of Assay Sensitivity. Report SPI/AMC 99-1/S499.

1869 DERMAL ONCOGENICITY STUDY OF BENZOYL PEROXIDE CARBOPOL GEL IN FISCHER 344 RATS.

L. C. Totman[1], R. L. Binder[2], S. J. Freeman[3], E. J. Winkelman[3], D. J. Minnema[4] and J. F. Nash[2]. *[1]Consumer Healthcare Products Association, Washington, DC, [2]Procter & Gamble Co., Cincinnati, OH, [3]SmithKline Beecham Consumer Healthcare, Parsippany, NJ and [4]Covance Laboratories, Inc., Vienna, VA.*

The oncogenic potential of benzoyl peroxide (BPO) was evaluated in male and female F344 rats by topical application in a carbopol gel vehicle. BPO gel was applied at doses of 5, 15 and 45 mg BPO/rat once daily for 104 weeks to a 3.5-x-5-cm treatment area on the dorsal skin. A discontinuous-treatment group received the high dose (45 mg of BPO/day) for 52 weeks and the vehicle for the remainder of the study. Vehicle-only and no-treatment groups served as controls. Rats were sacrificed at 52 (interim-sacrifice) or 104 weeks, and necropsies were performed. Treatment with BPO carbopol gel had no effect on survival, body weights, food consumption or gross pathology. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were mild-to-moderate degrees of hyperkeratosis, acanthosis, sebaceous gland hyperplasia and chronic subepidermal inflammation in all treatment groups. These effects were observed in the interim-sacrifice groups and in the rats sacrificed at 104 weeks. In the discontinuous-treatment group, hyperkeratosis was present approximately twice as frequently as in the controls, which suggests a partial but incomplete recovery. No findings indicative of oncogenicity resulted from daily topical application of BPO gel at doses up to 45 mg of BPO/day for 104 weeks. The absence of any skin tumors is particularly important considering the presence of chronic hyperplasia and other skin effects indicative of reaching the maximum tolerated dose in this study. These data together with chronic studies in mice support the conclusion that BPO lacks carcinogenic potential.

1870 DERMAL ONCOGENICITY STUDY OF BENZOYL PEROXIDE CARBOPOL GEL IN B6C3F1 MICE.

R. L. Binder[1], L. C. Totman[2], S. J. Freeman[3], E. J. Winkelman[3], D. J. Minnema[4] and J. F. Nash[1]. *[1]Procter & Gamble Co., Cincinnati, OH, [2]Consumer Healthcare Products Association, Washington, DC, [3]SmithKline Beecham Consumer Healthcare, Parsippany, NJ and [4]Covance Laboratories, Inc., Vienna, VA.*

The oncogenic potential of benzoyl peroxide (BPO) was evaluated in male and female B6C3F1 mice by topical application in a carbopol gel vehicle for 104 weeks. BPO gel was applied at doses of 1, 5 and 25 mg of BPO/mouse once daily to a 2-x-3-cm treatment area on the dorsal skin. A discontinuous-treatment group received 25 mg of BPO/day for 52 weeks and the carbopol gel vehicle for the remainder of the study. Vehicle-only and no-treatment groups served as controls. Mice were sacrificed at 52 (interim-sacrifice) or 104 weeks, and necropsies were performed. The high dose (25 mg of BPO/day) exceeded the maximum tolerated dose (MTD), in that it caused treatment-site ulceration, and was lowered to 15 mg of BPO/day at week 57. Because ulceration also occurred in mice receiving 15 mg/day, BPO treatment of the high-dose mice was suspended and vehicle was administered from week 93 until the end of the study. BPO had no effect on survival, body weights, food consumption or gross pathology, except for treatment-site ulceration in the high-dose group. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were dose-dependent induction of acanthosis, hyperkeratosis, sebaceous gland hyperplasia and subepidermal inflammation was evident at the interim sacrifice as well as at the terminal sacrifice in groups continuously treated with BPO. Ulceration, observed grossly, was confirmed microscopically. No findings indicative of oncogenicity resulted from topical application of BPO gel in any of the treatment groups. Importantly, 5 mg of BPO, administered in carbopol gel daily for 104 weeks, produced skin effects consistent with reaching but not exceeding the MTD. These data further support the conclusion that BPO lacks carcinogenic potential.